EFFICACY OF ANTIMICROBIAL COMPOUNDS IN *Camellia sinensis* FOR RED ROOT PATHOGEN

CLAMENT CHIN FUI SEUNG

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DR. Ivy Wong Nyet Kui
PENYELIA

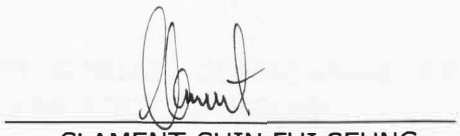
Dr. Ivy Wong Nyet Kui
Ketua Program Bioteknologi
Sekolah Sains Dan Teknologi
Universiti Malaysia Sabah

Tarikh: 14-08-2010

Tarikh: 14/8/2010

DECLARATION

I hereby declare that the materials in this thesis are my own except for quotations, excerpts, summaries and references, which have been duly acknowledged.



08 August 2010

CLAMENT CHIN FUI SEUNG

PC2006-8636

CERTIFICATION

NAME	:	**CLAMENT CHIN FUI SEUNG**
MATRIC NO	:	**PC2006-8636**
TITLE	:	**EFFICACY OF ANTIMICROBIAL COMPOUNDS IN *Camellia sinensis* FOR RED ROOT PATHOGEN**
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VIVA DATE	:	**08 JUNE 2010**

DECLARED BY

1. **MAIN SUPERVISOR**
   DR. IVY WONG NYET KUI

Dr. Ivy Wong Nyet Kui
Ketua Program Bioteknologi
Sekolah Sains Dan Teknologi
Universiti Malaysia Sabah

2. **CO-SUPERVISOR**
   PROF. MADYA DR. MARKUS ATONG

PROF. MADYA DR. MARKUS ATONG
TIMBALAN DEKAN (PENYELIDIKAN & INOVASI)
SEKOLAH PERTANIAN LESTARI
UNIVERSITI MALAYSIA SABAH

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ABSTRACT

EFFICACY OF ANTIMICROBIAL COMPOUNDS IN *Camellia sinensis* FOR RED ROOT PATHOGEN

Red root disease of tea is a primary root disease in Sabah Tea Plantation. The causal pathogen was successfully isolated and was identified as *Schizopora flavipora*, which previously known as *Poria hypolateritia*. The crude ethanolic extracts of tea (*Camellia sinensis* var. *assamica*) seed, root, stem and leaf; as well as their correspondence fractions (hexane, dichloromethane, ethyl acetate, butanol, and aqueous) were screened *in vitro* for antifungal activities using disc diffusion technique. Extracts from tea leaf and young immature green stem were more fungistatic than the root and seed extracts. Hexane, dichloromethane and ethyl acetate fractions obtained from the ethanolic leaf extracts by liquid-liquid extractions were more effective than the butanol or aqueous fraction when tested against the pathogen. The compounds that are responsible for fungitoxicity were identified by thin layer chromatography and high performance chromatography to be essential oils, alkaloids, phenolic acids and catechins. The constituents of essential oil that are responsible for fungitoxicity were identified to be geraniol, nerol, nerolidol, linalool, linalool oxide and benzyl acetate by the vanillin-sulphuric acid reagent, with the first two being the more potential, having a minimum inhibitory concentration (MIC) value of 0.25 $mg.mL^{-1}$. The groups of non-volatile active compounds were identified as caffeine, gallic acid, catechins and gallic acid esterified catechins, with the first two having the lowest MIC value (0.10 $mg.mL^{-1}$). The general distribution pattern of the non-volatile active compounds on thirteen parts of the tea plant were further determined and were found to contain highest in leaf bud and lowest in old root cortex. In this study, neither phytoalexins production nor active compounds accumulation was recorded after elicitation treatment using 0.1% (w/v) chitosan on leaves and roots of two years old tea seedlings. The study demonstrated that leaf extracts of *C. sinensis* exhibit strong fungitoxicity against *Schizopora flavipora*, and have potential to be used as mulching materials in infested soils through integrated pest management (IPM) strategy in organic farming, as well as formulating into biopesticide product for the control of red root disease in conventional farming.

Keywords: alkaloids, essential oils, polyphenols, red root disease, tea

ABSTRAK

Penyakit Akar merah teh merupakan penyakit penting di Ladang Teh Sabah. Patogen penyebab penyakit telah berjaya diisolat dan dikenalpasti sebagai Schizopora flavipora. Pada pemencilan awal, ekstrak etanol kasar dari biji benih, akar, batang dan daun teh (Camellia sinensis var. *assamica); serta hasil pecahannya (heksana, diklorometana, etil asetat, butanol, dan akueus) telah dipencilkan secara in vitro terhadap aktiviti antikulat menggunakakn teknik resapan disk. Ekstrak daun dan batang hijau muda teh didapati lebih berkesan dalam merencat pertumbuhan kulat berbanding dengan ekstrak akar dan biji benih. Pecahan heksana, diklorometana dan etil asetat yang terhasil daripada penyarian cecair-ceciar terhadap ekstrak etanol daun adalah lebih berkesan berbanding dengan pecahan butanol atau akueus apabila diuji terhadap patogen tersebut. Kehadiran kumpulan sebatian yang bertanggungjawab terhadap keracunan kulat telah dikenal pasti sebagai minyak pati, alkaloid, asid fenolik dan katekin melalui kromatografi lapisan nipis dan kromatografi cecair prestasi tinggi. Unsur-unsur minyak pati yang bertangungjawab terhadap keracunan kulat telah dikenal pasti sebagai geraniol, nerol, nerolidol, linalool, linalool oxide dan benzyl acetate melalui reagen vanillin-asid sulfurik yang mana unsur pertama dan kedua didapati lebih berpotensi, dengan nilai kepekatan perencat minimum (MIC) sebanyak 0.25 mg.mL^{-1}. Unsur aktif dalam sebatian tak meruap telah dikenal pasti sebagai kafeina, asid galik, katekin dan katekin terester asid galik, dengan unsur pertama dan kedua mempunyai nilai MIC yang paling rendah (0.10 mg.g^{-1}). Corak taburan am bagi unsur aktif tidak meruap pada tiga belas bahagian pokok teh telah ditentukan selanjutnya dan didapati kandungannya tinggi di tunas daun dan rendah di korteks akar tua. Berdasarkan kepada keputusan kajian ini, kedua-dua penghasilan fitoaleksin dan pengumpulan unsur aktif pada daun dan akar anak pokok teh berumur dua tahun tidak dicatatkan selepas rawatan elisitasi menggunakan 0.1% kitosan. Kajian tersebut telah menunjukkan ekstrak daun C. sinensis mempamerkan keracunan kulat yang kuat ke atas Schizopora flavipora, dan berpotensi digunakan sebagai bahan sungkupan pada tanah yang dikerumuni melalui strategik pengurusan perosak secara bersepadu (IPM) pada tanaman organik, serta diformulasikan kepada biopestisida produk untuk penggunaan di penanaman konvensional.*

Kata kunci: *alkaloid, minyak pati, polifenol, penyakit akar merah, teh*

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LIST OF SYMBOLS AND ABBREAVIATIONS

ACN	Acetonitrile
NH_4^+	Ammonium ion
ANOVA	Analysis of variances
BA	Benzyl acetate
BuOH	Butanol
Caf	Caffeine
C	Carbon
CO_2	Carbon dioxide
C	(+)-Catechin
CG	(-)-Catechin gallate
cm	Centimeter
CFS	Conventional farming system
r^2	Correlation coefficient value
°C	Degree Celsius
DCM	Dichloromethane
/	Divided by
DW	Dry weight
EC	(-)-Epicatechin
ECG	(-)-Epicatechin gallate
EGC	(-)-Epigallocatechin
EGCG	(-)-Epigallocatechin gallate
=	Equals to
↔	Equilibrium
EtOH	Ethanol
EtOAc	Ethyl acetate
FW	Fresh weight
GA	Gallic acid
GC	(-)-Gallocatechin
GCG	(-)-Gallocatechin gallate

GC-MS	Gas chromatography mass spectrometer
$R\text{-}NH_2$	General formula of amino in organic chemistry
R-OH	General formula of alcohols in organic chemistry
G	Geraniol
g	Gram
ha	Hectare
HEX	n-Hexane
HPLC	High performance liquid chromatography
h	Hour
H^+	Hydrogen ion
OH^{-1}	Hydroxy ion
IPM	Integrated pest management
kg	Kilogram
Km	Kilometer
LB	Leaf bud
≤	Less or equal to
L	Linalool
LO	Linalool oxide
L	Liter
m	Meter
MeOH	Methanol
CH_3	Methyl group
µg	Microgram
$\mu g.g^{-1}$	Microgram per gram
$\mu g.mL^{-1}$	Microgram per milliliter
µL	Microliter
µm	Micrometer
mAU	Milli absorbance units
mg	Milligram
$mg.g^{-1}$	Milligram per gram
$mg.mL^{-1}$	Milligram per milliliter
mL	Milliliter
mm	Millimeter

MIC	Minimum inhibition concentration
MIC_{50}	Minimum inhibitory concentration for 50% reduce in growth
MIC_{90}	Minimum inhibitory concentration for 90% reduce in growth
-	Minus
min	Minute
≥	More or equal to
>	More than
nm	Nanometer
N	Nerol
Nd	Nerolidol
NO_3^-	Nitrate ion
N	Nitrogen
<	Not more than
OL	Old leaf
ORB	Old root barks
ORC	Old root cortex
OSB	Old stem bark
OSC	Old stem cortex
1-D	One dimensional
OFS	Organic farming system
O_2	Oxygen
ppm	Part per million
%	Percent
H_3PO_4	Phosphoric acid
+	Plus
±	Plus or minus
RRD	Red root disease
R_f	Retention factor
R_t	Retention time
ST	Sabah Tea
STP	Sabah Tea Plantation
n	Sample size
SC	Seed coat

P	Significant value
mm^2	Square millimeter
SPSS	Statistical Analysis for Social Science
H_2SO_4	Sulphuric acid
TA	Tannic acid
TRI	Tea research institute
LD_{50}	The median lethal dose in toxicology
TF	Theaflavin
Tg	Theogallin
Tp	Theophylline
TLC	Thin layer chromatography
$T.ha^{-1}$	Ton per hectare
2-D	Two-dimensional
UV-VIS	Ultraviolet and visible
v/v	Volume to volume
H_2O	Water
w/v	Weight to volume
YBS	Young brown stem
YGS	Young green stem
YL	Young leaf
YR	Young root